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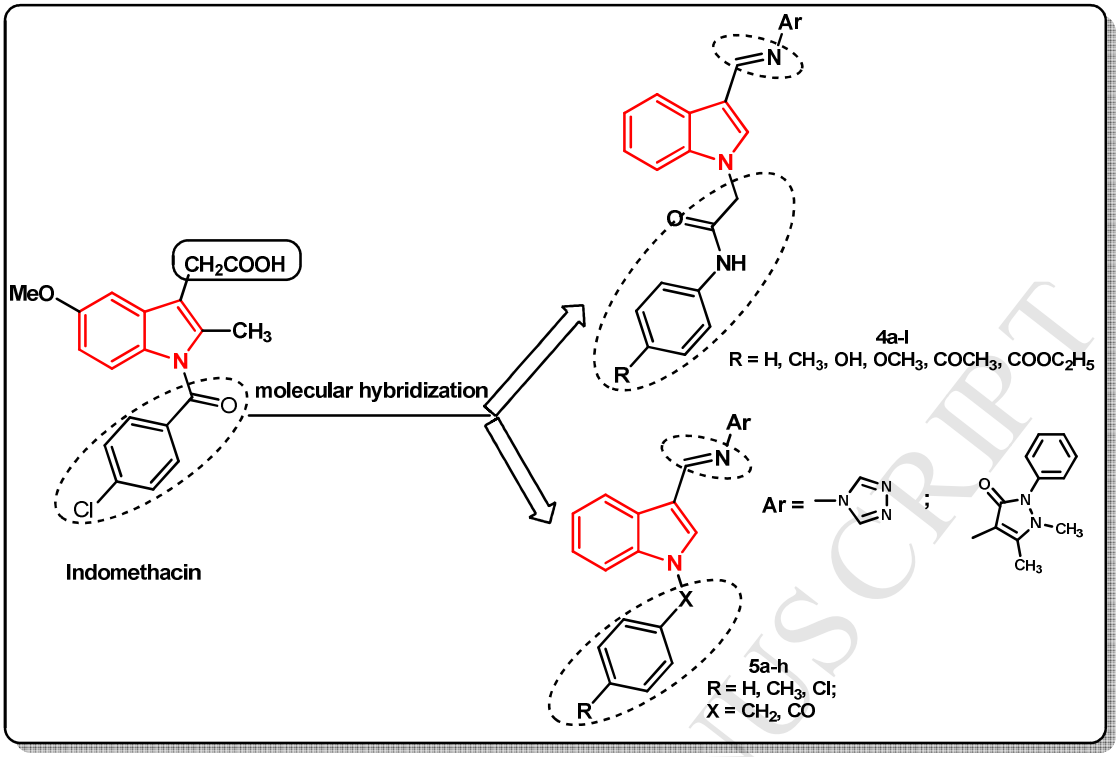

Novel *N*-substituted indole Schiff bases as dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase enzymes: synthesis, biological activities *in vitro* and docking study

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Abstract

Two new series of *N*-substituted indole derivatives **4a-l** and **5a-h** were synthesized. Their chemical structures were confirmed using spectroscopic tools including IR, ^{1}H NMR, ^{13}C NMR mass spectroscopy and elemental analyses. The results showed no significant cytotoxic activity on either cancer or normal human cells. Anti-inflammatory activity for all target compounds was evaluated *in-vitro*. Compounds **5a-h** were found to have better anti-inflammatory activity than **4a-l**. The inhibitory activity of COX-2 and 5-LOX were tested for **5a-h**. Three compounds, **5c**, **5d** and **5f** showed excellent COX-2 inhibitory activity with IC_{50} ranging from 0.98 to 1.23 µM compared to the reference celecoxib (1.54 µM). These compounds had a resonable selectivity index between 7.03-8.05. Additionally, *p*-methylbenzoyl derivative **5g** (IC_{50} = 5.78 µM) had superior 5-LOX inhibitory activity, higher than quercetin. **5e** was close to quercetin in its LOX inhibitory activity. Compounds **5a-h** were docked inside the active site of COX-2 and 5-LOX enzymes.

Keywords: Indole derivatives; Antiproliferative activity; Anti-inflammatory activity; Molecular docking study; Cyclooxygenase enzymes.

1. Introduction

Inflammation is one of the earliest signs of many well defined diseases and characterized by symptoms including pain, redness, heat, and swelling. Search for new drugs that relieve inflammation with high safety profiles is thus still a major target of drug therapy [1].

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used for the treatment of inflammatory symptoms [2]. These act by inhibiting the biotransformation of the polyunsaturated fatty acid, arachidonic acid (AA) which is a membrane bound phospholipid to prostaglandins (PGs), prostacyclin (PGI2) and thromboxane A2 (TXA2) *via* cyclooxygenase (COX) enzymes (COX-1, 2, 3) [3-5].

Non selective NSAIDs such as aspirin, phenazone and indomethacin and, selective COX-2 inhibitors like celecoxib, rofecoxib and valdecoxib produce undesirable side effects either by decreasing the cytoprotective action of the constitutive COX-1 isoform in the gastrointestinal tract (GIT) leading to ulcerogenic, hepatic and renal toxicity or by increasing cardiovascular disorders of selective COX-2, the inducible isoform, due to imbalance in the COX pathway. This is caused by decreasing PGI2 concentration which is a potent vasodilator and antithrombotic - relative to an increase in the prothrombotic - TXA2 leading to cardiovascular side effects which was the main cause of their withdrawal from the market [6-14].

In addition to the COX-mediated pathway, AA is also metabolized to leukotrienes (LTs) *via* lipoxygenase (LOX) enzymes (5-, 8-, 12-, 15-LOX). Two of these have been found in humans. 5-LOX is associated with inflammation, bronchoconstriction, hypersensitivity, anaphylaxis and asthma. 15-LOX is involved in atherosclerosis [6,15-18].

Accordingly, designing drugs with dual inhibitory activity for COX/LOX enzymatic pathways offers new options for developing more effective anti-inflammatory agents with improved safety profile.

A large number of studies [19-23] hve used the indole ring based NSAIDs as in indomethacin, as a target to improve their COX-2 selectivity and reduce their ulcerogenic side effects attributed to their high COX-1 selectivity and the acidic properties of the drug. Knaus *et al.* [24], prepared a series of indole derivatives

substituted at N1 and C3 positions. These compounds showed excellent COX-2 inhibitory activity.

Moreover, synthesis of compounds with the pyrazole ring and *N*-hydroxyamide (tepoxalin) [25] showed 5-LOX activity. Dual activity against COX and 5-LOX was observed in compounds containing the vinyl bridge between the phenyl and the heterocyclic five membered ring as in drabufelone [18, 26]. Although, tenidap [3] -COX/5LOXinhibitor- was more effective than traditional NSAIDs such as piroxicam and diclofenac in rheumatic arthritis, it was short lived, in the Europe market due to the liver and kidney toxicity of the reactive oxidative metabolite of thiophene moiety [3,27,28] (Fig. 1).

[Please, insert Fig. 1 about here]

Given these findings and as a continuation of our earlier work on the development of selective COX-2 inhibitors [29], we now report the design and synthesis of two series of novel indole containing compounds, **4a-l** and **5a-h** substituted at the N-1 and C-3 positions with: i) *N*-(4-substituted phenyl)-acetamide derivatives of indole N-1 in compounds **4a-l** similar to 5-LOX amide pharmacophore, ii) the chlorobenzoyl moiety of indomethacin in position 1, which is important in anti-inflammatory activity [22], is maintained in **5d, 5h**, replaced with benzoyl in **5b**, **5f** or with *p*-tolylbenzoyl part in **5c, 5g** or modified to benzyl in **5a**, **5e**, iii) the small methyl group at C-2 was omitted completely, iv) the ulcerative carboxylic acid group -CH_2COOH at C-3 in indomethacin was replaced with heteroaryl ring (pyrazole in **4a-f** and **5a-d** or triazole **4g-l** and **5e-h**) through azomethine linkage to mimic compounds with dual COX/5-LOX activity (Fig. 2).

[Please, insert Fig. 2 about here]

We evaluated the *in-vitro* anti-inflammatory activity of the two series **4a-l** and **5a-h**. COX-1, COX-2 and 5-LOX inhibitory activities of the most active compounds **5a-h** were determined. The plausible binding interactions of these compounds inside COX-1, COX-2 and 5-LOX active sites were explored using molecular modeling studies.

2. Results and Discussion

2.1.Chemistry

The synthesis of the target compounds is depicted in Scheme 1. The starting compounds, *N*-[(1*H*-Indol-3-yl)methylene]-4*H*-1,2,4-triazol-4-amine (**3a**) and 4-{[(1*H*-Indol-3-yl)methylene]amino}-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**3b**) were prepared *via* reacting indole-3-carbaldehyde (**1**) and the appropriate primary amine namely, 4-amino-1,2,4-triazole and 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (4-aminophenazone) in absolute ethanol at reflux temperature in 77-89% yield. The C=O group of indole-3-carbaldehyde (**1**) disappeared in the IR spectra of **3a&b**.

^{1}H NMR spectra of **3a&b** showed a singlet peak at δ 7.86 and 8.03 due to N=CH proton and D_2O exchangeable singlet signal of indole NH at δ 11.61 and 11.99. Additionally, one singlet signal at δ 9.07 due to two triazole protons in the **3a** spectrum, and two singlet signals at δ 2.47 and 3.10 due to CH_3 and N-CH_3 protons in **3b** spectrum. Moreover, the ^{13}C NMR spectra of **3a&b** exhibited a peak at δ 152.83, 155.32 revealed to -N=CH, as well as C-3, C-5 of triazole ring in **3a** at δ 139.27 and three different peaks at δ 10.48, 36.42 and 160.80 due to CH_3, N-CH_3, C=O of phenazone ring in **3b** spectrum.

The mass spectra of compounds **3a&b** showed a molecular ion peak at *m/z* 211 (77.09%) and 330 (27.84%) due to (M), respectively.

For preparing indolyl-*N*-substituted acetamide derivatives **4a-l** in (28-48%) yield, compounds **3a&b** reacted with 4-substituted phenylcarbonylmethyl chlorides that were prepared by following the same reaction conditions of reported methods [30-32], in DMF and sodium hydride (NaH) as a base. The formations of compounds **4a-l** were confirmed by ^{1}H NMR that showed a singlet signal due to CH_2 protons of acetamide moiety at δ 5.08-5.29 and only one exchangeable singlet signal at δ 10.23-10.85 of NH proton of acetamide and not of the parent indole compounds **3a&b**. This aside, the ^{13}C NMR spectra of compounds **4a-l** exhibited C=O group of acetamide part at δ 165.21-167.67.

On the other hand, the *N*-benzyl (**5a & 5e**) and *N*-benzoyl (**5b-d** & **5f-h**) derivatives were prepared by reaction of **3a&b** with benzyl chloride, benzoyl chloride, 4-methylbenzoyl chloride and 4-chlorobenzoyl chloride in the presence of NaH in DMF at room temperature for 24 h. The formation of compounds **5a-h** (yield; 43% - 86%) was confirmed by ^{1}H NMR that revealed the disappearance of the D_2O

exchangeable singlet signal of **3a&b** and the appearance of a singlet signal at δ 5.47 and 5.56 corresponding to benzyl CH_2 protons in **5a** & **5e**. ^{13}C NMR spectra of **5b-d** & **5f-h** showed the presence of the benzoyl C=O group at δ 167.77-168.76.

[Please, insert Scheme 1 about here]

2.2. Anti-inflammatory activity

2.2.1. In vitro anti-inflammatory assay

The *in vitro* anti-inflammatory properties of the indole derivatives were studied using ELISA in pretreated Human Umbilical Vein Endothelial Cells (HUVEC) where these compounds were able to inhibit NF-κB. E-selectin (ELAM) expression was induced by TNFα, which is indicative of NF-κB activation. The observed reduction of ELAM expression on treatment of HUVECs with 50 µM of the first series of indole substances was 15% decrease by **4d** and **4f**; the more effective second series caused significant decrease with 30 µM or 50 µM of indole derivative **5c** (decrease of 33 %). This correlates with data from enzyme inhibition of COX-2. Slight inhibition of ELAM expression and also cell viability was measured for all tested substances. The results confirmed that the NF-κB pathway was targeted by the indole derivatives (Fig. 3a, b).

[Please, insert Fig. 3 about here]

2.2.2. Inhibition of COX-1, COX-2 and 5-LOX – biological assays

The COX activity assay kit measures the peroxidase activity of cyclooxygenase enzyme isoforms. The target of the *in-vitro* biological activity assay was to explore the ability of tested compounds to inhibit both ovine COX-1 and COX-2 using a colorimetric enzyme immunoassay (EIA) kit by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm. The kit includes isozyme-specific inhibitors for distinguishing COX-2 activity from COX-1 activity. The COX assay allows the screening of a vast number of inhibitors and saves much time. The potency of tested compounds was determined as the concentration causing 50% enzyme inhibition (IC_{50}). Also, the COX-2 selectivity index (SI values) which is defined as IC_{50} (COX-1)/IC_{50} (COX-2) was calculated and compared with that of the standard drug celecoxib. Compounds **5a-h** were tested and the data are listed in Table 1.

The results showed that compounds **5a-h** were weak inhibitors of the COX-1 enzyme. They showed (IC_{50} = 7.65 – 11.23 μM) compared with indomethacin (IC_{50} = 0.63 μM). Compound **5c** with (IC_{50} = 7.89 μM) similar to that of celebrex (IC_{50} = 7.59 μM) for COX-1 enzyme inhibition, exhibited better COX-2 enzyme inhibitory activity (IC_{50} = 0.98 μM) than celecoxib (IC_{50} = 1.54 μM). Moreover, compounds **5f** and **5d** are classified as excellent COX-2 inhibitors with IC_{50} = 1.06 and 1.23 μM, respectively. Compound **5h** also showed good inhibitory activity against COX-2 and its IC_{50} equals 1.74 μM. The other compounds **5a**, **5b**, **5e** and **5g** had moderate COX-2 inhibitory activity with IC_{50} ranging from 2.01-2.61 μM. A common feature of the three most active compounds **5c**, **5f** and **5d** was the substitution of indole - NH with a benzoyl moiety. Generally, when fixing benzoyl moiety, compounds bearing tirazole nucleus at C-3 is more active than those with phenazone part . Thus, **5c** > **5g** and **5d** > **5h**. Between triazole series, *N*-benzyl indole **5a** and *N*-unsubstituted benzoyl indole **5b** showed the lowest IC_{50} (2.01 and 2.19 μM, sequentially). Accordingly, the least active compound was **5e** with both phenazone part and benzyl moiety.

So, the results showed COX-2 selectivity indices in the range 4.30 to 8.05 for **5a-h**. Within the synthesized compounds, compounds **5c**, **5d**, **5f** and **5h** showed a better COX-2 selectivity index (S.I. = 5.31 – 8.05) compared to celecoxib (S.I. = 4.92). Compounds **5g** and **5b** showed a COX-2 selectivity index close to celecoxib (S.I. = 4.84 and 4.75, respectively). The smallest COX-2 selectivity index was obtained from **5a** and **5e** (S.I = 4.58 and 4.30, sequentially).

For *in vitro* lipoxygenase (LOX) inhibitor screening, the assay kit detects and measures the hydroperoxides produced in the lipoxygenation reaction using a purified LOX enzyme. The detection reaction is equally sensitive to hydroperoxides at various positions within the fatty acid, and will work with fatty acids of any carbon length. It is thus a general detection method for LOX and can be used to screen libraries of compounds for those which inhibit LOX enzymes. 5-LOX enzyme is distinguished from other LOXs that introduce hydroperoxidate to lineolate and arachdonate substrates. Using the enzyme assay kit, the concentration of hydroxyperoxidase was measured. Quercetin was used as a reference drug in this study. The data are listed in Table 1.

Regarding 5-LOX inhibitory activity, compound **5g** was the highest 5-LOX inhibitor with (IC_{50} = 5.78 μM) compared with that of quercetin (IC_{50} = 5.96 μM). Compound **5e** showed inhibitory activity against 5-LOX enzyme (IC_{50} = 5.98 μM), most similar to quercetin. The most active compound **5g**, has a phenazone nucleus at position C-3 in its structure. This combination favours lipoxygenase inhibitory activity.

[Please, insert Table 1 about here]

2.2.3. In vitro cytotoxic activity

Fourteen tested indole derivatives from the first series were evaluated for their cytotoxicity on three cancer cell lines (CEM, MCF7, HeLa) and normal human fibroblasts. These substances were mostly inactive, except for **3a** (strong cytotoxicity on HeLa) and **4l** with slight cytotoxicity on all tested cell lines (Table 2).

Eight indole derivatives from second series were tested for cytotoxicity in human umbilical vein endothelial cells (HUVEC). After 24 h, these derivatives showed no cytotoxicity towards HUVECs except for **5a** IC_{50} 14.9 ± 3.0 (Table 2).

[Please, insert Table 2 about here]

2.3. Docking study

In a docking study, all new designed compounds were docked using AutoDock Vina into COX-2 and 5-LOX receptors. However in the case of the *N*-substituted indole derivatives synthetized in this study, we found that these compounds showed two distinct binding poses: (i) one group of compounds (count of heavy atom around 25) with the best binding free energy characterized by binding inside the active site of receptor **5a – 5d** and in particular, H-bond interaction between ligand and amino acid HIS90 in the COX-2 receptor, and (ii) a second group of large compounds (count of heavy atom more than 30) **5e – 5h**. The problem here was that they had to attain the active site of both receptors (Table 3). Docking of compound 5h inside the active site of 5-LOX is illustrated in Fig. 4.

[Please, insert Table 3 and Fig. 4 about here]

3. Conclusion

In summary, we synthesized new indole **3a&b** and *N*-substituted indole derivatives **4a-l** and **5a-h**. These derivatives were tested for both cytotoxic and anti-inflammatory activities. There was no significant cytotoxic activity on either cancer or normal human cells. The first series of indole derivatives **4a-l** showed slight anti-inflammatory activity *in vitro*. In the second series, **5a-h**, **5c** was the most active substance inhibiting expression of cytokine E-selectin. The latter compounds **5a-h** were evaluated against COX-1, COX-2 and 5-LOX enzymes. *P*-Methylbenzoyl derivative **5c** was the most active inhibitor of COX-2 enzyme showing IC_{50} = 0.98 µM and selectivity index equal to 8.05, while *p*-chlorobenzoyl derivative **5h** was the most potent selective 5-LOX inhibitor in this series with IC_{50} equals to 9.11 µM. Molecular docking showed that all new compounds bound to selected receptors. It is very important to follow the count of atoms of each ligand as the size of ligand plays an important role in the final conformation to active site and binding energy.

4. Experimental

4.1. Chemistry

Melting points were determined using a Griffin apparatus and were uncorrected. IR spectra were recorded on a Shimadzu IR-435 spectrophotometer using KBr discs and values were represented in cm^{-1}. ^{1}H NMR and ^{13}C NMR (DEPT-Q) were carried out using the Bruker instrument at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectrophotometer, (Faculty of Pharmacy, Beni Suef University, Beni Suef, Egypt), in DMSO-d_6, D_2O using TMS as an internal standard and chemical shifts were recorded in ppm on the δ scale using DMSO-d_6 (2.5) as a solvent. Coupling constant (*J*) values were estimated in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet, t, triplet; q, quartet; dd, doublet of doublet; m, multiplet. The electron impact (EI) mass spectra were recorded on a Hewlett Packard 5988 spectrometer (Palo Alto, CA). Microanalysis was performed for C, H, N on Perkin-Elmer 2400 at the Microanalytical center, Cairo University, Egypt and was within $\pm$ 0.4% of theoretical values. Analytical thin layer chromatography (TLC): pre-coated plastic sheets, 0.2 mm silica gel with UV indicator (Macherey-Nagel) was employed routinely to follow the course of reactions and to check the purity of products. All other reagents, solvents and compound **1** were purchased from the Aldrich Chemical Company (Milwaukee, WI) and, were used without further purification.

*4.1.1 General procedure for synthesis of compounds **3a&b***

A mixture of indole-3-carboxaldehyde (**1**) (300 mg, 1 mmol) and the appropriate primary amine **2a** or **2b** (1.2 mmol) in absolute ethanol (10 mL) was refluxed for 5-7 h (monitored by TLC). The precipitate that formed on hot was filtered off, dried and recrystallized from 95% ethanol to afford **3a&b**.

*4.1.1.1. N-[(1H-Indol-3-yl)methylene]-4H-1,2,4-triazol-4-amine (**3a**).* White crystals; 89% yield; mp 291-293°C; IR (KBr) 3126 (NH), 1600 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 7.21-7.29 (m, 2H, indole H-5, H-6), 7.53 (d, *J* = 8.00 Hz, 1H, indole H-7), 8.03 (s, 1H, *N=CH*), 8.21 (d, *J* = 8.00 Hz, 1H, indole H-4), 9.07 (s, 2H, triazole H-3, H-5), 9.15 (s, 1H, indole H-2), 11.99 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 110.36 (indole C-3), 112.87 (indole C-7), 121.87 (indole C-5), 122.17 (indole C-6), 123.75 (indole C-4), 124.45 (indole C-3a), 134.93 (indole C-2), 137.71 (indole C-7a), 139.27 (triazole C-3, C-5), 155.32 (CH=N); EIMS (m/z) 212 (M+1, 13.92%), 211 ($M^{+\cdot}$, 77.09%), 129 (100%). Anal. Calcd for $C_{11}H_9N_5$: C, 62.55; H, 4.29; N, 33.16. Found: C, 62.37; H, 4.15; N, 33.11.

*4.1.1.2. 4-{[(1H-Indol-3-yl)methylene]amino}-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (**3b**).* Yellow crystals; 77% yield; mp 271-273°C; IR (KBr) 3159 (NH), 2977, 2921 (CH-aliphatic), 1623 (C=O), 1594 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.47 (s, 3H, CH_3), 3.10 (s, 3H, N-CH_3), 7.15-7.23 (m, 2H, indole H-4, phenyl H-4), 7.34 (t, *J* = 7.20 Hz, 1H, indole H-5), 7.40 (d, *J* = 7.60 Hz, 2H, phenyl H-2, H-6), 7.45 (d, *J* = 7.6 Hz, 1H, indole H-7), 7.52 (t, *J* = 7.6 Hz, 2H, indole H-3, H-5), 7.86 (s, 1H, N=CH), 8.43 (d, J = 7.6 Hz, 1H, indole H-4), 9.76 (s, 1H, indole H-2), 11.61 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.48 (CH_3), 36.42 (N-CH_3), 112.30 (indole C-7), 116.34 (pyrazole C-4), 118.94 (indole C-3), 121.05 (indole C-5), 122.54 (indole C-6), 123.02 (indole C-4), 124.37 (phenyl C-2, C-6), 125.14 (indole C-3a), 126.82 (phenyl C-4), 129.54 (phenyl C-3, C-5), 132.02 (indole C-2), 135.43 (phenyl C-1), 137.71 (indole C-7a), 151.44 (pyrazole C-5), 152.83 (CH=N), 160.80 (C=O); EIMS (m/z) 331 (M+1, 7.67%), 330 ($M^{+\cdot}$, 27.84%), 56 (100%). Anal. Calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96. Found: C, 72.57; H, 5.25; N, 16.99.

*4.1.2. General procedure for synthesis of compounds **4a-l***

A solution of **3a** or **3b** (1 mmol) in DMF (5 mL) was added to the solution of sodium hydride (NaH) (1.5 mmol) in DMF (5 mL) and the reaction mixture was stirred for 30 min. at room temperature. A solution of the appropriate 2-chloro-*N*-phenylacetamide derivatives (1.5 mmol) in DMF (5 mL) was added, and the reaction mixture was stirred for 24 h at room temperature. The solution was poured onto ice-cold water. The formed precipitate was filtered off, dried and recrystallized from 95% ethanol to furnish **4a-l**.

*4.1.2.1. 2-{3-[((4H-1,2,4-Triazol-4-yl)imino)methyl]-1H-indol-1yl}-N-phenylacetamide **(4a)***. White powder; 45% yield; mp 275-277°C; IR (KBr) 3262 (NH) 1693 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 5.24 (s, 2H, CH_2), 7.08 (t, *J* = 7.2 Hz, 1H, phenyl H-4), 7.26-7.35 (m, 4H, indole H-5, H-6 and phenyl H-3, H-5), 7.55-7.61 (m, 3H, indole H-7 and phenyl H-2, H-6), 8.09 (s, 1H, N=CH), 8.24 (d, *J* = 7.2 Hz, 1H, indole H-4), 9.10 (s, 2H, triazole H-3, H-5), 9.13 (s, 1H, indole H-2), 10.51 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 49.83 (CH_2), 109.90 (indole C-3), 111.25 (indole C-7), 119.61 (phenyl C-2, C-6), 122.23 (indole C-5), 122.39 (indole C-6), 123.94 (indole C-4), 124.11 (phenyl C-4), 124.94 (indole C-3a), 129.36 (phenyl C-3, C-5), 138.30 (indole C-7a), 138.75 (indole C-2), 139.08 (phenyl C-1), 139.30 (triazole C3, C-5), 154.83 (CH=N), 166.01 (C=O); EIMS (m/z) 345 (M+1, 12.99%), 344 ($M^{+\cdot}$, 18.83%), 129 (100%). Anal. Calcd for $C_{19}H_{16}N_6O$: C, 66.27; H, 4.68; N, 24.40. Found: C, 66.42; H, 4.53; N, 24.51.

*4.1.2.2. 2-{3-[((4H-1,2,4-triazol-4-yl)imino)methyl]-1H-indol-1yl}-N-(p-tolyl)acetamide **(4b)***. White powder; 47% yield; mp 272-274°C; IR (KBr) 3256 (NH) 1695 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.25 (s, 3H, CH_3), 5.22 (s, 2H, CH_2), 7.13 (d, *J* = 8 Hz, 2H, phenyl H-3, H-5), 7.26-7.34 (m, 2H, indole H-5, H-6), 7.49 (d, *J* = 8 Hz, 2H, phenyl H-2, H-6), 7.57 (d, *J* = 8 Hz, 1H, indole H-7), 8.09 (s, 1 H, CH=N), 8.23 (d, *J* = 7.6 Hz, 1H, indole H-4), 9.10 (s, 2H, triazole C-3, C-5), 9.14 (s, 1H, indole H-2), 10.48 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 20.91(CH_3), 49.80 (CH_2), 109.85 (indole C-3), 111.27 (indole C-7), 119.61 (phenyl C-2, C-6), 122.22 (indole C-5), 122.37 (indole C-6), 123.92 (indole C-4), 124.92 (indole C-3a), 129.71 (phenyl C-3, C-5), 133.03 (phenyl C-1), 136.60 (indole C-7a), 138.28 (phenyl C-4), 138.77 (indole C-2), 139.30 (triazole C-3, C-5), 154.83 (CH=N), 165.75 (C=O); EIMS (m/z) 359 (M+1, 13.13%),

358 ($M^{+\cdot}$, 14.75%), 77 (100%). Anal. Calcd for $C_{20}H_{18}N_6O$: C, 67.02; H, 5.06; N, 23.45. Found: C, 66.88; H, 5.13; N, 23.42.

4.1.2.3. *2-{3-[((4H-1,2,4-triazol-4-yl)imino)methyl]-1H-indol-1yl}-N-(p-hydroxyphenyl) acetamide (**4c**).* Yellow powder; 39% yield; mp 230-232°C; IR (KBr) 3323 (OH), 3257 (NH) 1700 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 5.17 (s, 2H, CH_2), 6.72 (d, *J* = 8 Hz, 2H, phenyl H-3, H-5), 7.29-7.55 (m, 6H, indole H-5, H-6, H-7, phenyl H-2, H-6, CH=N), 8.13 (d, *J* = 7.6 Hz, 1 H, indole H-4), 9.11 (s, 1H, indole H-2), 9.28 (s, 2H, triazole H-3, H-5), 9.96 (s, 1H, OH, D_2O exchangeable), 10.48 (s, 1H, NH, D_2O exchangeable);^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 49.95 (CH_2), 109.82 (indole C-7), 115.67 (phenyl C-3, C-5), 117.82 (indole C-3), 121.47 (phenyl C-2, C-6), 122.39 (indole C-5), 123.93 (indole C-6), 124.11 (indole C-4), 124.97 (indole C-3a) 130.64 (phenyl C-1), 138.15 (indole C-7a), 138.78 (indole C-2), 142.76 (triazole C-3, C-5), 154.10 (phenyl C-4), 154.86 (CH=N), 165.21 (C=O); EIMS (m/z) 361 (M+1, 15.28%), 360 ($M^{+\cdot}$, 21.35%), 155 (100%). Anal. Calcd for $C_{19}H_{16}N_6O_2$: C, 63.32; H, 4.48; N, 23.32. Found: C, 63.58; H, 4.13; N, 23.55.

4.1.2.4. *2-{3-[((4H-1,2,4-triazol-4-yl)imino)methyl]-1H-indol-1yl}-N-(p-methoxyphenyl) acetamide (**4d**).* Light violet powder; 46% yield; mp 276-278°C; IR (KBr) 3267 (NH) 1695 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 3.71 (s, 3H, OCH_3), 5.20 (s, 2H, CH_2), 6.90 (d, *J* = 8.8 Hz, 2H, phenyl H-3, H-5), 7.26-7.35 (m, 2H, indole H-5, H-6), 7.52 (d, *J* = 8.8 Hz, 2H, phenyl H-2, H-6), 7.57 (d, *J* = 7.6 Hz, 1H, indole H-7), 8.09 (s, 1H, CH=N), 8.24 (d, *J* = 7.6 Hz, 1H, indole H-4), 9.11(s, 2H, triazole H-3, H-5), 9.14 (s, 1H, indole H-2), 10.37 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 51.97 (CH_2), 57.82 (OCH_3), 112.06 (indole C-3), 113.45 (indole C-7), 116.66 (phenyl C-3, C-5), 123.37 (phenyl C-2, C-6), 124.45 (indole C-5), 124.60 (indole C-6), 126.15 (indole C-4), 127.14 (indole C-3a), 134.38 (phenyl C-1), 140.48 (indole C-7a), 140.99 (indole C-2), 141.52 (triazole C-3, C-5), 157.04 (CH=N), 158.11 (phenyl C-4), 167.67 (C=O); EIMS (m/z) 374 ($M^{+\cdot}$, 5.07%), 122 (100%). Anal. Calcd for $C_{20}H_{18}N_6O_2$: C, 64.16; H, 4.85; N, 22.45. Found: C, 63.99; H, 4.73; N, 22.27.

*4.1.2.5. 2-{3-[((4H-1,2,4-triazol-4-yl)imino)methyl]-1H-indol-1yl}-N-(p-acetylphenyl) acetamide (**4e**).* Yellow powder; 32% yield; mp 286-288°C; IR (KBr) 3231 (NH),

1694, 1665 (2C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.50 (s, 3H, CH_3), 5.29 (s, 2H, CH_2), 7.28-7.33 (m, 2H, indole H-5, H-6), 7.59 (d, *J* = 8 Hz, 1H, indole H-7), 7.74 (d, *J* = 8.8 Hz, 2H, phenyl H-3, H-5), 7.96 (d, *J* = 8.8 Hz, 2H, phenyl H-2, H-6), 8.10 (s, 1H, CH=N), 8.25 (d, *J* = 6.8 Hz, 1H, indole H-4), 9.11(s, 2H, triazole H-3, H-5), 9.13 (s, 1H, indole H-2), 10.85 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 26.91 (CH_3), 49.82 (CH_2), 109.97 (indole C-3), 111.24 (indole C-7), 118.96 (phenyl C-2, C-6), 122.32 (indole C-5), 122.40 (indole C-6), 124.03 (indole C-4), 124.87 (indole C-3a), 130.09 (phenyl C-3, C-5), 132.49 (indole C-7a), 138.30 (phenyl C-4), 138.77 (indole C-2), 139.32 (triazole C-3, C-5), 143.31 (phenyl C-1), 154.85 (CH=N), 166.73 (NHC=O), 197.16 (C=O); EIMS (m/z) 386 ($M^{+\cdot}$, 0.16%), 155 (100%). Anal. Calcd for $C_{21}H_{18}N_6O_2$: C, 65.27; H, 4.70; N, 21.75. Found: C, 65.39; H, 4.70; N, 21.55.

*3.1.2.6. Ethyl 4-{2-[3-(((4H-1,2,4-triazol-4-yl)imino)methyl)-1H-indol-1yl]-acetamido}benzoate (**4f**).* Yellow powder; 47% yield; mp 245-247°C; IR (KBr) 3269 (NH) 1704, 1668 (2C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.30 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 4.28 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 5.28 (s, 2H, CH_2), 7.26-7.35 (m, 2H, indole H-5, H-6), 7.59 (d, *J* = 8 Hz, 1H, indole H-7), 7.74 (d, *J* = 8.4 Hz, 2H, phenyl H-2, H-6), 7.94 (d, *J* = 8.4 Hz, 2H, phenyl H-3, H-5), 8.09 (s, 1H, CH=N), 8.24 (d, *J* = 7.6 Hz, 1H, indole H-4), 9.11 (s, 2H, triazole H-3, H-5), 9.13 (s, 1H, indole H-2), 10.84 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 14.65 (CH_3), 49.83 (CH_2), 60.99 (CH_2CH_3), 109.98 (indole C-3), 111.27 (indole C-7), 119.07 (phenyl C-2, C-6), 122.28 (indole C-5), 122.39 (indole C-6), 124.00 (indole C-4), 124.88 (phenyl C-4), 125.07 (indole C-3a), 130.85 (phenyl C-3, C-5), 138.32 (indole C-7a), 138.74 (indole C-2), 139.31 (triazole C-3, C-5), 143.35 (phenyl C-1), 154.82 (CH=N), 165.74 (C=O), 166.73 (NHC=O); EIMS (m/z) 417 (M+1, 22.60%), 416 ($M^{+\cdot}$, 28.08%), 91 (100%). Anal. Calcd for $C_{22}H_{20}N_6O_3$: C, 63.45; H, 4.84; N, 20.18. Found: C, 63.39; H, 4.71; N, 20.25.

*4.1.2.7. 2-{3-[((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-4-yl)imino)methyl]-1H-indol-1yl}-N-phenylacetamide (**4g**).* Yellow powder; 39% yield; mp 228-230°C; IR (KBr) 3163 (NH) 1695, 1627 (2C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.50 (s, 3H, CH_3), 3.12 (s, 3H, N-CH_3), 5.14 (s, 2H, CH_2), 7.07 (t, *J* = 6.8 Hz, 1H, anilide phenyl H-4), 7.20-7.30 (m, 2H, indole H-5, H-6), 7.32-7.37 (m, 3H, phenyl H-2, H-4, H-6), 7.41 (d, *J* = 7.6 Hz, 2H, anilide phenyl H-3, H-5), 7.48-7.55 (m, 3H,

phenyl H-3, H-5 and indole H-7), 7.60 (d, *J* = 8 Hz, 2H, anilide phenyl H-2, H-6), 7.91 (s, 1H, CH=N), 8.44 (d, *J* = 7.6 Hz, 1H, indole H-4), 9.75 (s, 1H, indole H-2), 10.46 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.50 (CH_3), 36.37 (N-CH_3), 49.72 (CH_2), 110.70 (indole C-7), 115.79 (pyrazole C-4), 118.76 (indole C-3), 119.60 (indole C-5), 121.45 (anilide phenyl C-4), 122.67 (anilide phenyl C-2, C-6), 123.24 (phenyl C-4), 124.05 (indole C-6), 124.46 (phenyl C-2, C-6), 125.65 (indole C-3a), 126.89 (indole C-4), 129.34 (phenyl C-3, C-5), 129.54 (anilide phenyl C3, C-5), 135.38 (anilide phenyl C-1), 136.07 (indole C-2), 138.26 (phenyl C-1), 139.12 (indole C-7a), 151.53 (pyrazole C-5), 152.11 (CH=N), 160.76 (C=O), 166.31 (NHC=O); EIMS (m/z) 464 (M+1, 4.46%), 463 ($M^{+\cdot}$, 13.62%), 56 (100%). Anal. Calcd for $C_{28}H_{25}N_5O_2$: C, 72.55; H, 5.44; N, 15.11. Found: C, 72.60; H, 5.43; N, 15.01.

*4.1.2.8. 2-{3-[((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-4-yl)imino)methyl]-1H-indol-1yl}-N-(p-tolyl)acetamide (**4h**).* Buff powder; 41% yield; mp 258-260°C; IR (KBr) 3259 (NH) 1660, 1627 (2C=O) cm^{-1};^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.25 (s, 3H, *p*-CH_3), 2.50 (s, 3H, CH_3), 3.12 (s, 3H, N-CH_3), 5.11 (s, 2H, CH_2), 7.12 (d, *J* = 7.6 Hz, 2H, anilide phenyl H-3, H-5), 7.20-7.28 (m, 2H, indole H-5, H-6), 7.34 (t, *J* = 7.6 Hz, 1H, phenyl H-4), 7.41 (d, *J* = 8 Hz, 2H, phenyl H-2, H-6), 7.48-7.55 (m, 5H, indole H-7, phenyl H-3, H-5 and anilide phenyl H-2, H-6), 7.90 (s, 1H, CH=N), 8.45 (d, *J* = 7.6 Hz, 1H, indole H-4), 9.76 (s, 1H, indole H-2), 10.36 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.49 (CH_3), 20.90 (*p*-CH_3), 36.35 (N-CH_3), 49.70 (CH_2), 110.70 (indole C-7), 115.78 (pyrazole C-4), 118.77 (indole C-3), 119.64 (anilide phenyl C-2, C-6), 121.44 (indole C-5), 122.67 (phenyl C-4), 123.24 (indole C-6), 124.47 (phenyl C-2, C-6), 125.66 (indole C-3a), 126.90 (indole C-4), 129.54 (phenyl C-3, C-5), 129.71 (anilide phenyl C-3, C-5), 133.01 (anilide phenyl C-1), 135.37 (anilide phenyl C-4), 136.06 (indole C-2), 136.60 (phenyl C-1), 138.26 (indole C-7a), 151.51 (pyrazole C-5), 152.12 (CH=N), 160.76 (C=O), 166.05 (NHC=O); EIMS (m/z) 478 (M+1, 0.83%), 477 ($M^{+\cdot}$, 2.25%), 56 (100%). Anal. Calcd for $C_{29}H_{27}N_5O_2$: C, 72.94; H, 5.70; N, 14.66. Found: C, 72.75; H, 5.73; N, 14.56.

*4.1.2.9. 2-{3-[((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-4-yl)imino)methyl]-1H-indol-1yl}-N-(p-hydroxyphenyl)acetamide (**4i**).* Grey powder; 28% yield; mp 229-231°C; IR (KBr) 3430 (OH), 3258 (NH) 1681, 1617 (2C=O) cm^{-1}; ^1H NMR (400

MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CH_3), 3.11 (s, 3H, N-CH_3), 5.08 (s, 2H, CH_2), 6.71-6.98 (m, 3H, anilide phenyl H-3, H-5, phenyl H-4), 7.24-7.51 (m, 9H, indole H-5, H-6, H-7, phenyl H-2, H-3, H-5, H-6, anilide phenyl H-2, H-6), 7.90 (s, 1H, CH=N), 8.44-8.45 (m, 1H, indole H-4), 9.28 (s, 1H, OH, D_2O exchangeable), 9.76 (s, 1H, indole H-2), 10.23 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.44 (CH_3), 36.26 (N-CH_3), 49.67 (CH_2), 110.66 (indole C-7), 115.66 (anilide phenyl C-3, C-5), 115.72 (pyrazole C-4), 118.68 (indole C-3), 121.87 (anilide phenyl C-2, C-6), 122.19 (indole C-5), 122.65 (phenyl C-4), 123.27 (indole C-6), 124.57 (phenyl C-2, C-6), 125.66 (indole C-3a) 126.99 (indole C-4), 129.57 (phenyl C-3, C-5), 130.69 (anilide phenyl C-1), 135.27 (phenyl C-1), 136.01 (indole C-2), 138.18 (indole C-7a), 151.40 (pyrazole C-5), 152.13 (CH=N), 154.17 (anilide phenyl C-4), 160.72 (C=O), 165.56 (NHC=O); EIMS (m/z) 480 (M+1, 1.52%), 479 ($M^{+\cdot}$, 4.26%), 56 (100%). Anal. Calcd for $C_{28}H_{25}N_5O_3$: C, 70.13; H, 5.25; N, 14.60. Found: C, 70.52; H, 5.37; N, 14.48.

*4.1.2.10. 2-{3-[((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-4-yl)imino)methyl]-1H-indol-1yl}-N-(p-methoxyphenyl)acetamide (**4j**).* Orange powder; 42% yield; mp 220-222°C; IR (KBr) 3275 (NH) 1669, 1627 (2C=O) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.49 (s, 3H, CH_3), 3.11 (s, 3H, N-CH_3), 3.71 (s, 3H, O-CH_3), 5.10 (s, 2H, CH_2), 6.90 (d, *J* = 8.8 Hz, 2H, anilide phenyl H-3, H-5), 7.15-7.22 (m, 2H, indole H-5, H-6), 7.26 (t, *J* = 8.4 Hz, 1H, phenyl H-4), 7.34 (d, *J* = 6.8 Hz, 2H, phenyl H-2, H-6), 7.37-7.53 (m, 5H, indole H-7, phenyl H-3, H-5 and anilide phenyl H-2, H-6), 7.90 (s, 1H, CH=N), 8.45 (d, *J* = 7.6 Hz, 1H, indole H-4), 9.76 (s, 1H, indole H-2), 10.32 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.50 (CH_3), 36.37 (N-CH_3), 49.67 (CH_2), 55.61 (OCH_3), 110.71 (indole C-7), 115.77 (anilide phenyl C-3, C-5), 118.79 (pyrazole C-4), 121.16 (indole C-3), 123.01 (anilide phenyl C-2, C-6), 123.23 (indole C-5), 124.36 (phenyl C-4), 124.54 (indole C-6), 125.67 (phenyl C-2, C-6), 126.88 (indole C-3a), 129.54 (indole C-4), 132.24 (phenyl C-3, C-5), 135.39 (anilide phenyl C-1), 136.08 (phenyl C-1), 137.71 (indole C-2), 151.52 (indole C-7a), 152.13 (pyrazole C-5), 152.82 (CH=N), 155.86 (anilide phenyl C-4), 160.76 (C=O), 165.76 (NHC=O); EIMS (m/z) 494 (M+1, 3.34%), 493 ($M^{+\cdot}$, 10.21%), 56 (100%). Anal. Calcd for $C_{29}H_{27}N_5O_3$: C, 70.57; H, 5.51; N, 14.19. Found: C, 70.43; H, 5.57; N, 14.08.

*4.1.2.11. N-(p-Acetylphenyl)-2-{3-[((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-4-yl)imino)methyl]-1H-indol-1yl}acetamide (**4K**).* Orange powder; 47% yield; mp 246-248°C; IR (KBr) 3257 (NH), 1673-1627 (3 C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.37 (s, 3H, CH_3), 2.49 (s, 3H, $COCH_3$), 3.12 (s, 3H, N-CH_3), 5.20 (s, 2H, CH_2), 7.08-7.26 (m, 2H, indole H-5, H-6), 7.35 (t, *J* = 6.8 Hz, 1H, phenyl H -4), 7.41 (d, *J* = 7.6 Hz, 2H, phenyl H-2, H-6), 7.46-7.54 (m, 3H, indole H-7, phenyl H-3, H-5), 7.75 (d, *J* = 8.4 Hz, 2H, anilide phenyl H-2, H-6), 7.92 (s, 1H, CH=N), 7.95 (d, *J* = 8.4 Hz, 2H, anilide phenyl H-3, H-5), 8.46 (d, *J* = 7.6 Hz, 1H, indole H-4), 9.77 (s, 1H, indole H-2), 10.84 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.49 (CH_3), 26.91 ($COCH_3$), 36.44 (N-CH_3), 49.74 (CH_2), 110.74 (indole C-7), 115.90 (pyrazole C-4), 118.76 (indole C-3), 118.93 (anilide phenyl C-2, C-6), 121.47 (indole C-5), 123.00 (phenyl C-4), 123.28 (indole C-6), 124.47 (phenyl C-2, C-6), 125.65 (indole C-3a), 126.89 (indole C-4), 129.54 (phenyl C-3, C-5), 130.07 (anilide phenyl C-3, C-5), 132.42 (phenyl C-1), 135.38 (anilide phenyl C-4), 136.02 (indole C-2), 138.33 (indole C-7a), 143.44 (anilide phenyl C-1), 151.53 (pyrazole C-5), 152.09 (CH=N), 160.76 (C=O), 167.05 (NHC=O), 197.01 (CH_3C=O); EIMS (m/z) 506 (M+1, 3.45%), 505 ($M^{+\cdot}$, 10.48%), 56 (100%). Anal. Calcd for $C_{30}H_{27}N_5O_3$: C, 71.27; H, 5.38; N, 13.85. Found: C, 70.98; H, 5.39; N, 13.74.

*4.1.2.12. Ethyl 4-{2-[3-(((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-4-yl)imino)methyl)-1H-indol-1yl]acetamido}benzoate (**4l**).* Buff powder; 48% yield; mp 246-248°C; IR (KBr) 3256 (NH), 1705-1628 (3C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 1.30 (t, *J* = 6.8 Hz, 3H, CH_2CH_3), 2.49 (s, 3H, CH_3), 3.10 (s, 3H, N-CH_3), 4.28 (d, *J* = 6.8 Hz, 2H, CH_2CH_3), 5.20 (s, 2H, CH_2), 7.24-7.27 (m, 2H, indole H-5, H-6), 7.34 (t, *J* = 6.4 Hz, 1H, phenyl H-4), 7.41 (d, *J* = 7.6 Hz, 2H, phenyl H-2, H-6), 7.51-7.52 (m, 3H, indole H-7 and phenyl H-3, H-5), 7.77 (d, *J* = 8 Hz, 2H, anilide phenyl H-2, H-6), 7.92 (s, 1H, CH=N), 7.95 (d, *J* = 8 Hz, 2H, anilide phenyl H-3, H-5), 8.48 (d, *J* = 6.8 Hz, 1H, indole H-4), 9.79 (s, 1H, indole H-2), 10.82 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.46 (CH_3), 14.64 (CH_2CH_3), 36.40 (N-CH_3), 49.73 (CH_2), 60.98 (CH_2CH_3), 110.71 (indole C-7), 115.91 (pyrazole C-4), 118.72 (indole C-3), 119.07 (anilide phenyl C-2, C-6), 121.05 (indole C-5), 121.49 (phenyl C-4), 122.68 (indole C-6), 124.51 (phenyl C-2, C-6), 125.02 (indole C-3a), 125.64 (anilide phenyl C-4), 126.93 (indole C-4),

129.54 (phenyl C-3, C-5), 131.99 (indole C-2), 135.33 (phenyl C-1), 135.99 (anilide phenyl C-3, C-5), 138.30 (indole C-7a), 143.42 (anilide phenyl C-1), 151.49 (pyrazole C-5), 152.10 (CH=N), 160.74 (C=O), 165.76 (*C*OOCH$_2$CH$_3$), 167.02 (NHC=O); EIMS (m/z) 536 (M+1, 1.33%), 535 ($M^{+\cdot}$, 3.83%), 56 (100%). Anal. Calcd for $C_{31}H_{29}N_5O_4$: C, 69.52; H, 5.46; N, 13.08. Found: C, 69.72; H, 5.21; N, 12.99.

*4.1.3. General procedure for synthesis of compounds **5a-h***

A solution of **3a** or **3b** (1 mmol) in DMF (5 mL) was added to a solution of sodium hydride (NaH) (1.5 mmol) in DMF (5 mL) and the reaction mixture was stirred for 30 min. at room temperature. Either benzyl chloride (0.18 g, 1.5 mmol), or the appropriate 4-substituted benzoyl chloride derivative (1.5 mmol), was added. The reaction mixture was stirred for 24 h at room temperature. The solvent was removed in vacuoand, ice-cold water was added. The formed precipitate was filtered off, dried and recrystallized from 95% ethanol to furnish **5a-h**.

*4.1.3.1. N-[(1-Benzyl-1H-indol-3-yl)methylene]-4H-1,2,4-triazol-4-amine (**5a**).* White crystals; 86% yield; mp 195-197^{o}C; IR (KBr) 1607 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 5.56 (s, 2H, CH$_2$), 7.24-7.37 (m, 7H, 5 benzyl-H and indole H-5, H-6), 7.61 (d, *J* = 7.6 Hz, 1H, indole H-7), 8.18 (s, 1H, N=CH), 8.23 (d, *J* = 7.6 Hz, 1H, indole H-4), 9.08 (s, 2H, triazole H-3, H-5), 9.11 (s, 1H, indole H-2); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 50.08 (CH$_2$), 109.85 (indole C-3), 111.72 (indole C-7), 122.40 (indole C-5), 122.45 (indole C-6), 124.05 (indole C-4), 125.13 (indole C-3a), 127.73 (phenyl C-4), 128.28 (phenyl C-2, C-6), 129.21 (phenyl C-3, C-5), 137.39 (indole C-7a), 137.52 (phenyl C-1), 137.63 (indole C-2), 139.29 (triazole C-3, C-5), 155.00 (CH=N); EIMS (m/z) 302 (M+1, 1.2%), 301 ($M^{+\cdot}$, 6.2%), 255 (100%). Anal. Calcd for $C_{18}H_{15}N_5$: C, 71.74; H, 5.02; N, 23.24. Found: C, 71.91; H, 7.99; N, 23.15.

*4.1.3.2. N-[(1-Benzoyl-1H-indol-3yl)methylene)-4H-1,2,4-triazol-4-amine (**5b**).* Yellow crystals; 53% yield; mp 215-217°C; IR (KBr) 1670 (C=O), 1632 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 7.48-7.56 (m, 2H, indole H-5, H-6), 7.66 (*t, J* = 8.0 Hz, *2H,* benzoyl H-3, H-5), 7.76 (t, *J* = 7.6 Hz, 1H, benzoyl H-4), 7.86 (d, *J* = 8 Hz, 2H, benzoyl H-2, H-6), 8.02 (s, 1H, N=CH), 8.34-8.38 (m, 2H, indole H-4, H-7), 9.10 (s, 2H, triazole H-3, H-5), 9.20 (s, 1H, indole H-2); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 115.53 (indole C-3), 116.53 (indole C-7), 122.82 (indole C-5), 125.50 (indole C-6),

126.70 (indole C-3a), 126.72 (indole C-4), 129.35 (benzoyl C-3, C-5), 129.75 (benzoyl C-2, C-6), 133.06 (benzoyl C-4), 133.69 (benzoyl C-1), 136.02 (indole C-2), 136.88 (indole C-7a), 139.19 (triazole C-3, C-5), 153.81 (CH=N), 168.69 (C=O); EIMS (m/z) 316 (M+1, 11.4%), 315 ($M^{+\cdot}$, 63.9%), 195 (100%). Anal. Calcd for $C_{18}H_{13}N_5O$: C, 68.56; H, 4.16; N, 22.21. Found: C, 68.47; H, 4.15; N, 22.11.

*4.1.3.3. N-[(1-(p-Methylbenzoyl)-1H-indol-3yl)methylene)-4H-1,2,4-triazol-4-amine (**5c**).* Orange crystals; 49% yield; mp 117-119 ^oC; IR (KBr) 1678 (C=O), 1612 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.42 (s, 3H, CH_3), 7.42-7.54 (m, 4H, indole H-5, H-6, phenyl H-3, H-5), 7.74 (d, *J* = 8.0 Hz, 2H, *p*-methylbenzoyl H-2, H-6), 8.00 (d, *J* = 8.0 Hz,1H, indole H-7), 8.04 (s, 1H, N=CH), 8.33 (d, *J* = 8.0 Hz, 1H, indole H-4), 9.10 (s, 2H, triazole H-3, H-5), 9.18 (s, 1H, indole H-2); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 21.55 (CH_3), 115.31 (indole C-3), 116.39 (indole C-7), 122.80 (indole C-5), 125.39 (indole C-6), 126.61 (indole C-4), 128.39 (indole C-3a), 129.58 (*p*-methylbenzoyl C-3, C-5), 129.87 (*p*-methylbenzoyl C-2, C-6), 130.65 (*p*-methylbenzoyl C-1), 136.09 (indole, C-2), 136.85 (indole, C-7a), 139.21 (triazole C-3, C-5), 143.56 (phenyl C-4), 153.79 (CH=N), 168.59 (C=O); EIMS (m/z) 330 (M+1, 20.2%), 329 ($M^{+\cdot}$, 100%). Anal. Calcd for $C_{19}H_{15}N_5O$: C, 69.29; H, 4.59; N, 21.26. Found: C, 69.37; H, 4.55; N, 20.98.

*4.1.3.4. N-[(1-(p-Chlorobenzoyl)-1H-indol-3yl)methylene)-4H-1,2,4-triazol-4-amine (**5d**).* Yellow crystals; 64% yield; mp 209-211^oC; IR (KBr) 1686 (C=O), 1628 (C=N) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 7.53-7.57 (m, 2H, indole H-5, H-6), 7.74 (d, *J* = 6.8 Hz, 2H, *p*-chlorobenzoyl H-3, H-5), 7.88 (d, *J* = 6.8 Hz, 2H, *p*-chlorobenzoyl H-2, H-6), 8.03 (s, 1H, N=CH), 8.34-8.38 (m, 2H, indole H-4, H-7), 9.10 (s, 2H, triazole H-3, H-5), 9.17 (s, 1H, indole H-2); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 115.69 (indole C-3), 116.55 (indole C-7), 122.83 (indole C-5), 125.58 (indole C-6), 126.73 (indole C-3a), 126.78 (indole C-4), 129.44 (*p*-chlorobenzoyl C-3, C-5), 131.71 (*p*-chlorobenzoyl C-2, C-6), 132.56 (*p*-chlorobenzoyl C-1), 136.03 (indole, C-2), 136.85 (indole, C-7a), 137.88 (*p*-chlorobenzoyl C-4), 139.19 (triazole C-3, C-5), 153.73 (CH=N), 167.78 (C=O); EIMS (m/z) 349 ($M^{+\cdot}$, 5.1%), 91 (100%). Anal. Calcd for $C_{18}H_{12}ClN_5O$: C, 61.81; H, 3.46; N, 20.02 Found: C, 62.17; H, 3.35; N, 20.11.

*4.1.3.5. 4-{[(1-Benzyl-1H-indol-3yl)methylene]amino}-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (**5e**).* Yellow crystals; 68% yield; mp 174-176 °C; IR (KBr) 1644 (C=O), 1604 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.47 (s, 3H, CH_3), 3.11 (s, 3H, N-CH_3), 5.47 (s, 2H, CH_2), 7.21-7.29 (m, 2H, indole H-5, phenyl H-4), 7.31-7.37 (m, 6H, benzyl H-3, H-4, H-5, indole H-6, phenyl H-3, H-5), 7.40 (d, *J* = 7.6 Hz, 2H, benzyl H-2, H-6), 7.50-7.55 (m, 3H, indole H-7, phenyl H-2, H-6), 8.05 (s, 1H, N=CH), 8.44 (d, *J* = 8.8 Hz, 1H, indole H-4), 9.75 (s, 1H, indole H-2); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.39 (CH_3), 36.22 (N-CH_3), 49.88 (CH_2), 111.16 (indole C-7), 115.83 (pyrazole C-4), 118.62 (indole C-3), 121.49 (indole C-5), 122.71 (phenyl C-4), 123.29 (phenyl C-2, C-6), 124.66 (benzyl C-4), 125.85 (indole C-3a), 127.08 (indole C-6), 127.63 (indole C-4), 128.06 (benzyl C-2, C-6), 129.10 (phenyl C-3, C-5), 129.23 (benzyl C-3, C-5), 129.60 (phenyl C-2, C-6), 134.91 (indole C-2), 135.25 (phenyl C-1), 137.48 (indole C-7a), 137.93 (benzyl C-1), 151.31 (pyrazole C-5), 152.13 (CH=N), 160.69 (C=O); EIMS (m/z) 421 (M+1, 3.1%), 420 ($M^{+\cdot}$, 9.2%), 304 (100%). Anal. Calcd for $C_{27}H_{24}N_4O$: C, 77.12; H, 5.75; N, 13.32. Found: C, 77.02; H, 5.86; N, 13.17.

*4.1.3.6. 4-{[(1-Benzoyl-1H-indol-3yl)methylene]amino}-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (**5f**).* Orange crystals; 77% yield; mp 166-168°C; IR (KBr) 1673, 1642 (2C=O), 1602 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.50 (s, 3H, CH_3), 3.18 (s, 3H, N-CH_3), 7.37-7.39 (m, 3H, indole H-5, H-6, phenyl H-4), 7.46-7.55 (m, 4H, phenyl H-2, H-3, H-5, H-6), 7.61-7.64 (m, 2H, benzoyl H-3, H-5), 7.70 (t, *J* = 8.4 Hz, 1H, benzoyl H-4), 7.83 (d, *J* = 6.8 Hz, 2H, benzoyl H-2, H-6), 7.93 (s, 1H, N=CH), 8.32 (d, *J* = 9.2 Hz,1H, indole H-7), 8.60 (d, *J* = 9.2 Hz, 1H, indole H-4), 9.70 (s, 1H, indole H-2); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.46 (CH_3), 35.87 (N-CH_3), 115.50 (indole C-3), 116.26 (indole C-7), 117.56 (pyrazole C-4), 123.24 (indole C-5), 125.02 (phenyl C-4), 125.15 (phenyl C-2, C-6), 126.14 (indole C-6), 127.36 (indole C-4), 128.00 (indole C-3a), 129.29 (phenyl C-3, C-5), 129.64 (benzoyl C-3, C-5), 129.73 (benzoyl C-2, C-6), 132.69 (benzoyl C-1), 132.84 (indole C-2), 133.98 (phenyl C-1), 137.01 (indole C-7a), 143.26 (benzoyl C-4), 150.56 (CH=N), 153.56 (pyrazole C-5), 160.07 (C=O), 168.76 (benzoyl C=O)**;** EIMS (m/z) 434 ($M^{+\cdot}$, 3.6%), 310 (100%). Anal. Calcd for $C_{27}H_{22}N_4O_2$: C, 74.64; H, 5.10; N, 12.89. Found: C, 74.88; H, 5.36; N, 13.07.

*4.1.3.7. 1,5-Dimethyl-4-{[(1-(p-methylbenzoyl)-1H-indol-3yl)methylene]amino}-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (**5g**).* Orange crystals; 48% yield; mp 154-156°C; IR (KBr) 1664, 1642 (2C=O), 1603 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.44 (s, 3H, *p*-methylbenzoyl CH_3), 2.50 (s, 3H, CH_3), 3.17 (s, 3H, N-CH_3), 7.37-7.42 (m, 3H, indole H-5, H-6, phenyl H-4), 7.44-7.47 (m, 4H, *p*-methylbenzoyl H-3, H-5, phenyl H-2, H-6), 7.53 (t, *J* = 8.4 Hz, 2H, phenyl H-3, H-5), 7.73 (d, *J* = 8.00 Hz, 2H, *p*-methylbenzoyl H-2, H-6), 7.95 (s, 1H, N=CH), 8.28 (d, *J* = 8.8 Hz, 1H, indole H-7), 8.58 (d, *J* = 8.8 Hz, 1H, indole H-4), 9.70 (s, 1H, indole H-2); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.46 (CH_3), 21.63 (*p*-methylbenzoyl CH_3), 35.88 (N-CH_3), 116.18 (indole C-7), 117.59 (pyrazole C-4), 120.87 (indole C-3), 123.21 (indole C-5), 123.80 (phenyl C-4), 125.02 (phenyl C-2, C-6), 126.04 (indole C-6), 127.34 (indole C-4), 127.94 (indole C-3a), 129.64 (phenyl C-3, C-5), 129.82 (*p*-methylbenzoyl C-3, C-5), 129.99 (*p*-methylbenzoyl C-2, C-6), 131.06 (*p*-methylbenzoyl C-1), 132.76 (indole, C-2), 135.04 (phenyl C-1), 137.02 (indole C-7a), 143.25 (*p*-methylbenzoyl C-4), 150.62 (CH=N), 152.14 (pyrazole C-5), 160.09 (C=O), 168.64 (*p*-methylbenzoyl C=O); EIMS (m/z) 449 (M+1, 3.9%), 448 ($M^{+\cdot}$, 12.6%). Anal. Calcd for $C_{28}H_{24}N_4O_2$: C, 74.98; H, 5.39; N, 12.49. Found: C, 75.11; H, 5.28; N, 12.62.

*4.1.3.8. 4-{[(1-(p-Chlorobenzoyl)-1H-indol-3yl)methylene]amino}-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (**5h**).* Orange crystals; 43% yield; mp 202-204°C; IR (KBr) 1685, 1644 (2C=O), 1588 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.50 (s, 3H, CH_3), 3.17 (s, 3H, N-CH_3), 7.37-7.39 (m, 3H, indole H-5, H-6, phenyl H-4), 7.46, 7.47 (dd, *J* = 4Hz, 2H, 7.6 Hz, phenyl H-2, H-6), 7.53 (t, 2H, *J* = 8.4 Hz, phenyl H-3, H-5), 7.67 (d, 2H, *J* = 6.4 Hz, *p*-chlorobenzoyl H-3, H-5), 7.85 (d, *J* = 6.4 Hz, 2H, *p*-chlorobenzoyl H-2, H-6), 7.98 (s, 1H, N=CH), 8.32, 8.34 (dd, *J* = 1.2 Hz, 6.8 Hz, 1H, indole H-7), 8.59, 8.61 (dd, *J* = 1.2 Hz, 1H, 6.4 Hz, indole H-4), 9.70 (s, 1H, indole H-2); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 10.46 (CH_3), 35.87 (N-CH_3), 116.29 (indole C-7), 117.57 (pyrazole C-4), 121.27 (indole C-3), 123.28 (indole C-5), 125.00 (indole C-6), 125.22 (phenyl C-4), 126.17 (phenyl C-2, C-6), 127.32 (indole C-4), 128.04 (indole C-3a), 129.37 (phenyl C-3, C-5), 129.63 (*p*-chlorobenzoyl C-3, C-5), 131.69 (*p*-chlorobenzoyl C-2, C-6), 132.76 (indole, C-2), 132.80 (*p*-chlorobenzoyl C-1), 135.04 (phenyl C-1), 136.98 (indole C-7a), 137.59 (*p*-chlorobenzoyl C-4), 150.53 (CH=N), 152.20 (pyrazole C-5), 160.06 (C=O), 167.77

(*p*-chlorobenzoyl C=O); EIMS (m/z) 470 (M+2, 30.6%), 468 ($M^{+\cdot}$, 100%). Anal. Calcd for $C_{27}H_{21}ClN_4O_2$: C, 69.15; H, 4.51; N, 11.95. Found: C, 69.33; H, 4.58; N, 12.13.

4.2. Biological activity

4.2.1. In vitro anti-inflammatory activity:

ELISA of E-selectin was performed as described in a reported method [33]. Briefly, 1 × 10^4 HUVECs/well were seeded into 96-well plates and grown to confluence. 50 µM of indole derivatives were added 30 min prior to application of 10 ng/ml TNFα for another 4 h. The cells were then fixed and ELAM levels analysed by ELISA. At the same time, the compounds were analysed by Calcein AM assay to monitor non-specific substance toxicity.

4.2.2. In vitro cyclooxygenase (COX) inhibition assay

An enzyme immune assay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA) was used to test the ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and COX-2 (IC_{50} value, µM) according to a reported method [34, 35].

4.2.3. In vitro 5-lipoxygenase (LOX) inhibition assay

The ability of the test compounds listed in Table 1 to inhibit 5-LOX (IC_{50} value, µM) was determined using an enzyme immune assay (EIA) kit (catalogue no 760709, Cayman Chemical, Ann Arbor, MI, USA). Stock solutions were freshly prepared before use and buffer solution used (0.1 M Tris HCl, PH, 7.4). 10 µl of different compounds were prepared in dissolved at least the amount of DMSO and diluted with the stock solution concentrations of (0.001, 01, 1, 5, 10 µM) in a final volume of 210 µl. The IC_{50} of test compounds were determined according to the manufacturer's instructions and according to reported methods. [34, 35]

4.2.4. In vitro cytotoxicity:

4.2.4.1. Cell culture

Stock solutions (10 mmol/L) of the tested compounds were prepared by dissolving an appropriate quantity of each substance in dimethylsulfoxide (DMSO). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, penicillin and streptomycin were purchased from Sigma (MO, USA). Calcein AM was obtained from Molecular Probes (Life Technologies, CA, USA). The screening cell lines (T-lymphoblastic leukemia cell line CEM, breast carcinoma cell line MCF7, cervical carcinoma cell line HeLa and human fibroblasts BJ) were obtained from the

American Type Culture Collection (Manassas, VA, USA). All cell lines were cultured in DMEM medium (Sigma, MO, USA), supplemented with 10% fetal bovine serum, L-glutamine (2 mmol/l), penicillin (100 U) and streptomycin (100 µg/ml). The cell lines were maintained under standard cell culture conditions at 37 ºC and 5% CO_2 in a humid environment. Cells were subcultured twice or three times a week using the standard trypsinization procedure.

4.2.4.2. Calcein AM assay

Suspensions of tested cell lines (ca. 1.0×10^5 cells/ml) were placed in 96-well microtiter plates and after 24 h of stabilization (time zero) the tested compounds were added (in three 20 µl aliquots) in serially diluted concentrations in dimethylsulfoxide (DMSO). Control cultures were treated with DMSO alone. The final concentration of DMSO in the incubation mixtures never exceeded 0.6%. The test compounds were typically evaluated at six 3-fold dilutions and the highest final concentration was generally 50 µM. After 72 h incubation, Calcein AM solution (100 µl, Molecular Probes, Life Technologies, CA, USA) was added, and incubation was continued for another hour. The fluorescence of viable cells was then quantified using a Fluoroskan Ascent instrument (Labsystems, Finland). The percentage of surviving cells in each well was calculated by dividing the intensity of the fluorescence signals from the exposed wells by the intensity of signals from control wells and multiplying by 100. These ratios were then used to construct dose-response curves from which IC_{50} values, the concentrations of the respective compounds that were lethal to 50% of the tumor cells, were calculated.

4.3. Docking study

Docking was performed to obtain prediction of conformation and energy ranking between COX2 receptor (PDB ID: 4PH9) and 5-LOX receptor (PDBID: 3V99) and the designed set of molecules. The docking studies were carried out using AutoDock Vina 1.05 [16]. All 3D structures of ligands were obtained with Marvin 5.10.3, software which can be used for drawing, displaying and characterization of chemical structure, substructures and reactions. Polar hydrogens were added to all ligands and proteins with the AutoDock Tools (ADT) [36] program prior to docking with Autodock Vina program [37]. A grid box 25 Å in size was centered on the active site of protein. The exhaustiveness parameter was set to 20 (default 8). After docking, we compared the docked ligand with Ibuprofen (IBP) crystal-like poses of COX2

nature ligand and arachidonic Acid (ACD) nature ligand of 5-LOX receptor and the best crystal-like poses of each ligand were analyzed.

The active site of the COX-2 receptor is a bent narrow gap with two important polar amino acids around the active site. The first amino acid is ARG121 which is located deeper in the active site. This amino acid plays an important role in the interaction between receptor and nature ligand IBP. The second amino acid is HIS90 located at the entrance to the active site and this amino acid is important for new designed compounds (Fig. 5). The 5-LOX active site is a long curved tunnel with a key polar amino acid ARG182 near the surface of receptor (Fig. 5).

[Please, insert Fig. 5 about here]

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References

[1] L. Verma, S. Wakode, Synthesis and Characterisation of 3-Acetylindole Derivatives and Evaluation of Their Anti-Inflammatory and Anti-Microbial Activity, The Pharma Innovattion – Journal 2 (2013) 41-50.

[2] R. Paulose-Ram, R. Hirsch, C. Dillon, Q. Gu, Frequent monthly use of selected non-prescription and prescription non-narcotic analgesics among U.S. adults, Pharmacoepidemiol. Drug Saf. 14 (2005) 257-266.

[3] Y. Lai, L. Mab, W. Huang, X. Yu, Y. Zhang, H. Ji, J. Tian, Synthesis and biological evaluation of 3-[4-(amino/methylsulfonyl)phenyl]methylene-indolin-2-one derivatives as novel COX-1/2 and 5-LOX inhibitors, Bioorg. Med. Chem. Lett. 20 (2010) 7349–7353.

[4] A. Moreau, Q.H. Chen, P.N.P. Rao, E.E. Knaus, Design, synthesis, and biological evaluation of (E)-3-(4-methanesulfonylphenyl)-2-(aryl)acrylic acids as dual inhibitors of cyclooxygenases and lipoxygenases, Bioorg. Med. Chem. 14 (2006) 7716–7727.

[5] M.A. Chowdhury, K.R.A. Abdellatif, Y. Dong, D. Das, M. R. Suresh, E.E. Knaus, Synthesis of Celecoxib Analogues Possessing a *N*-Difluoromethyl-1,2-dihydropyrid-2-one 5-Lipoxygenase Pharmacophore: Biological Evaluation as Dual Inhibitors of Cyclooxygenases and 5-Lipoxygenase with Anti-Inflammatory Activity, J. Med. Chem. 52 (2009) 1525–1529.

[6] C. Charlier, C. Michaux, Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. Eur. J. Med. Chem. 38 (2003) 645-788.

[7] K. Seibert, Y. Zhang, K. Leahy, S. Hauser, J. Masferrer, W. Perkins, L. Lee, P.C. Isakson, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 12013-12017.

[8] T.D. Penning, J.J. Talley, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, L.F. Lee, J.W. Malecha, J.M. Miyashiro, R.S. Rogers, D.J. Rogier, S.S. Yu, G.D. Anderson, E.G. Burton, J.N. Cogburn, S.A. Gregory, C.M. Koboldt, W.E. Perkins, K. Seibert, A.W. Veenhuizen, Y.Y. Zhang, P.C. Isakson, Synthesis and Biological Evaluation of the 1,5-Diarylpyrazole Class of Cyclooxygenase-2 Inhibitors: Identification of 4-[5-(4-Methylphenyl)-3- (trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (SC-58635, Celecoxib), J. Med. Chem. 40 (1997) 1347-1365.

[9] P. Prasit, Z. Wang, C. Brideau, C.C. Chan, S. Charleson, W. Cromilish, D. Ethier, J.F. Evans, A.W. Ford-Hutchinson, J.Y. Gauthier, R. Gordon, J. Gyay, M. Gresser, S. Kargman, B. Kennedy, Y. Leblanc, S. Leger, J. Mancini, G.P. O'Neill, M. Ouellet, M.D. Percival, H. Perrier, D. Riendeau, I. Rodger, P. Tagari, M. Thierien, P. Vickers, E. Wong, L.-J. Xu, R.N. Young, R. Zamboni,The Discovery of Rofecoxib, [MK 966, Vioxx- , 4-(4' -methylsulfo nylphenyl)- 3- phenyl- 2(5*H*)- furanone, An Orally Active Cyclooxygenase-2 Inhibitor, Bioorg. Med. Chem. Lett. 9 (1999) 1773-1778.

[10] T. Grosser, S. Fries, G.A. Fitzgerald, Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. J. Clin. Invest. 116 (2006) 4-15.

[11] J. Badiger, K. Manjulatha, M. Girish, A. Sharif, M.G. Purohit, Synthesis and biological evaluation of some *N*-substituted indoles, Synthesis and biological evaluation of some *N*-substituted indoles, ARKIVOC xii (2009) 217-231.

[12] E.A. Meade, W.L. Smith, D.L. DeWitt, Differential Inhibition of Prostaglandin Endoperoxide Synthase (Cyclooxygenase) Isozymes by Aspirin and OtherN on-steroidal Antiinflammatory Drug, J. Biol. Chem. 268 (1993) 6610-6614.

[13] C. Charlier, C. Michaux, Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs, Eur. J. Med. Chem. 38 (2003) 645-659.

[14] L.J. Marnett, The COXIB Experience: A Look in the Rearview Mirror, Annu. Rev. Pharmacol. Toxicol. 49 (2009) 265–290.

[15] S. Kalva, L.M. Saleena, Pharmacophore Design, Homology and Docking Studies on 5-Lipoxygenase Inhibitors, International Conference on Bioscience, Biochemistry and Bioinformatics, 5 (2011) 368-372.

[16] L. Vila, Cyclooxygenase and 5-lipoxygenase pathways in the vessel wall: role in atherosclerosis, Med. Res. ReV. 24 (2004) 399–424.

[17] L. Zhao, C.D. Funk, Lipoxygenase pathways in atherogenesis, Trends Cardio Vasc. Med. 14 (2004) 191–195.

[18] S. Fiorucci, R. Meli, M. Bucci, G. Cirino, Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy, Biochem. Pharmacol. 62 (2001) 1433-1556.

[19] S. Khanna, M. Madan, A. Vangoori, R. Banerjee, R. Thaimattam, S.K.J. Basha, M. Ramesh, S.R. Casturi, M. Pal, Evaluation of glycolamide esters of indomethacin as potential cyclooxygenase-2 (COX-2) inhibitors
Bioorg. Med. Chem. 14 (2006) 4820-4833.

[20] A.S. Kalgutkar, B.C. Crews, S. Saleh, D. Prudhomme, L.J. Marnett, Indolyl esters and amides related to indomethacin are selective COX-2 inhibitors., Bioorg.
Med. Chem. 13 (2005) 6810-6822.

[21] M.A. Chowdhury, Z. Huang, K.R.A. Abdellatif, Y. Dong, G. Yu, C.A. Velgzquez, E.E. Knaus, Synthesis and biological evaluation of indomethacin analogs possessing a N-difluoromethyl-1, 2-dihydropyrid-2-one ring system: a search for novel cyclooxygenase and lipoxygenase inhibitors, Bioorg. Med. Chem. Lett. 20 (2010) 5776-5780.

[22] W. Hu, Z. Guo, F. Chu, A. Bai, X. Yi, G. Cheng, J. Li, Synthesis and biological evaluation of substituted 2-sulfonyl-phenyl-3-phenyl-indoles: a new series of selective COX-2 inhibitors, Bioorg. Med. Chem. 11 (2003) 1153-1160.

[23] B.P. Bandgar, R.J. Sarangdhar, S. Viswakarma, F.A. Ahamed, Synthesis and biological evaluation of orally active prodrugs of indomethacin, J. Med. Chem. 54 (2011) 1191-1201.

[24] J. Kaur, A. Bhardwaj, Z. Huang, E.E. Knaus, N-1 and C-3 substituted indole Schiff bases as selective COX-2 inhibitors: Synthesis and biological evaluation, Bioorg. Med. Chem. Lett. 22 (2012) 2154–2159.

[25] E.M.F. Muri, M.J. Nieto, R.D. Sindelar, J.S. Williamson, Hydroxamic acids as pharmacological agents, Curr. Med. Chem. 9 (2002)1631–1653.

[26] S.H. Hwang, A.T. Wecksler, K. Wagner, B.D. Hammock, Rationally Designed Multitarget Agents Against Inflammation and Pain, Current Medicinal Chemistry, 20 (2013) 1783-1799.

[27] S.D. Nelson, Structure toxicity relationships--how useful are they in predicting toxicities of new drugs, Adv. Exp. Med. Biol. 500 (2001) 33-43.

[28] H.G. Fouda, M.J. Avery, D. Dalvie, F.C. Falkner, L.S. Melvin, R.A. Ronfeld, Disposition and metabolism of tenidap in the rat, Drug Metab. Dispos. 25 (1997) 140-148.

[29] K.R.A. Abdellatif1, P.F. Lamie, H.A. Omar, 3-Methyl-2-phenyl-1-substituted-indole derivatives as indomethacin analogs: design, synthesis and biological evaluation as potential anti-inflammatory and analgesic agents, J. Enzyme Inhib. Med. Chem. 31 (2016) 318-324.

[30] N.R. Modi, R.J. Shah, M.J. Patel, M. Suthar, B.F. Chauhan, L.J. Patel, Design, synthesis, and QSAR study of novel 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-phenylacetamide derivatives as cytotoxic agents, Med. Chem. Res. 20 (2011) 615–625.

[31] H. Behbehani, H.M. Ibrahim, 4-Thiazolidinones in Heterocyclic Synthesis: Synthesis of Novel Enaminones, Azolopyrimidines and 2-Arylimino-5-arylidene-4-thiazolidinones, Molecules 17 (2012) 6363-6385.

[32] D. Chen, G. Shen*a*, W. Bao, An efficient cascade synthesis of various 2*H*-1,4-benzoxazin-3-(4*H*)-ones from *o*-halophenols and 2-halo-amides catalyzed by CuI, Org. Biomol. Chem. 7 (2009) 4067–4073.

[33] P.F. Lamie, J.N. Phillopes, A.O. El-Gendy, L. Rarova, J. Gruz, Design, synthesis and evaluation of novel phthalimide derivatives as *in Vitro* anti-microbial, anti-oxidant and anti-inflammatory agents. Molecules 20 (2015) 16620-16642.

[34] S. Yamamoto, Mammalian lipoxygenases: Molecular structures and functions. Biochim. Biophys.acta 1128 (1992) 117-131.

[35] B.J. Gaffney, Lipoxygenases: Structural principles and spectroscopy. Annu. Rev. Biophys. Biomol. Struct. 25 (1996) 431-459.

[36] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson. Autodock 4 and AutoDockTools 4: automated docking with selective receptor flexibility. J Comput Chem 16 (2009) 2785–2791.

[37] O. Trott, A.J. Olson. Software News and Update AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31 (2010) 455–461.

Tables, Figures and Schemes Legends:

Table 1: *In vitro* COX-1, COX-2 and 5- LOX inhibition, and selectivity index (SI) data for **5a-h** and the reference drugs celecoxib and quercetin.

Table 2: IC_{50} (μM) values obtained from Calcein AM assays on three tested cancer cell lines and normal human fibroblasts; means ±SD obtained from three independent experiments performed in triplicate.

Table 3: Resulting binding free energies for best poses.

Figure 1: Chemical structures of some traditional non-selective NSAIDs, some selective cyclooxygenase-2 (COX-2) inhibitor drugs, 5-LOX inhibitor, and dual COX/5-LOX inhibitory agent

Figure 2: Chemical structures of the traditional NSAID indomethacin (**3**), dual COX/5-LOX inhibitor darbufelone (**8**) and the designed indomethacin analogues **4a-l** and **5a-h**.

Figure 3a, b: E-Selectin (ELAM) expression in TNFα -induced HUVECs compared to cytotoxicity determined by Calcein AM. Curcumin (10 μM) was used as a positive control. **3a – 4l** was tested at 50 μM.

Figure 4: Compound **5h** around active site of 5-LOX receptor. It is possible to observe the binding using h-bond to Arg182.

Figure 5: Left 2D image is active site of COX2 receptor with nature ligand IBP and collection of amino acid 4Å around. Right image is 5-LOX receptor with ACD ligand and amino acids 4Å around.

Scheme 1: Synthesis of the target compounds 4a-l and 5a-h.

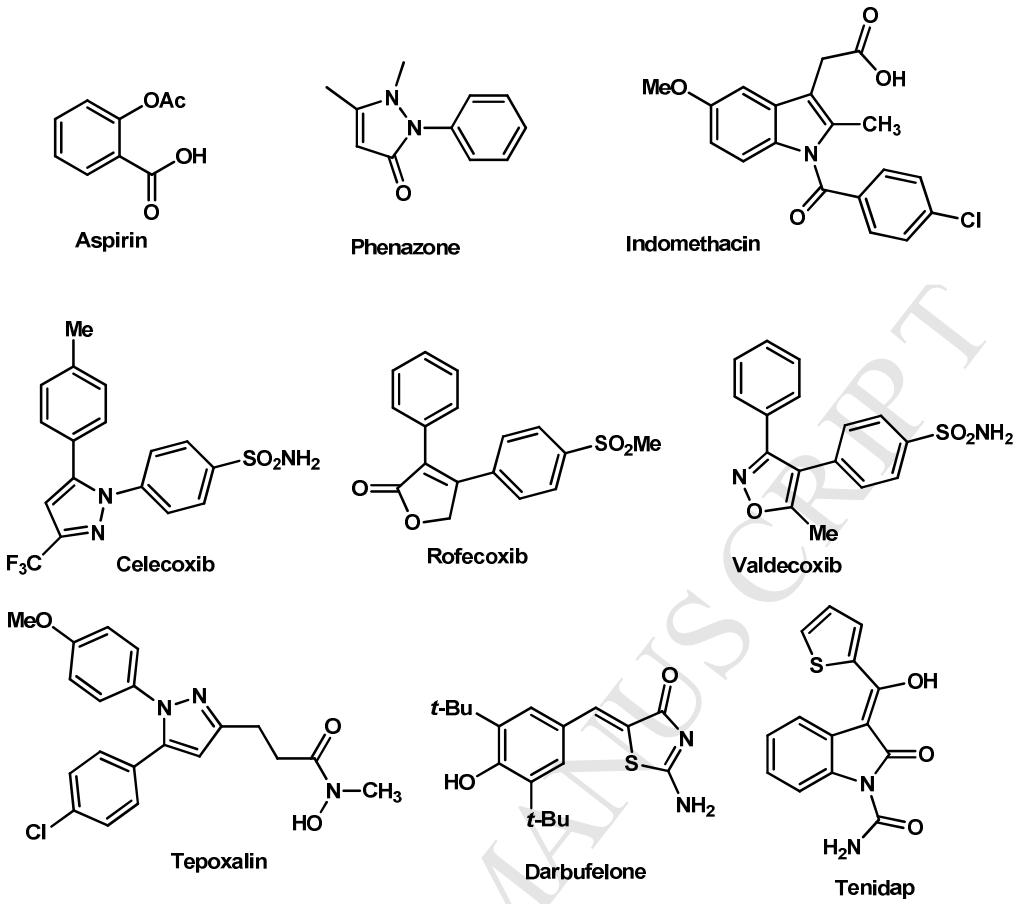


Figure 1

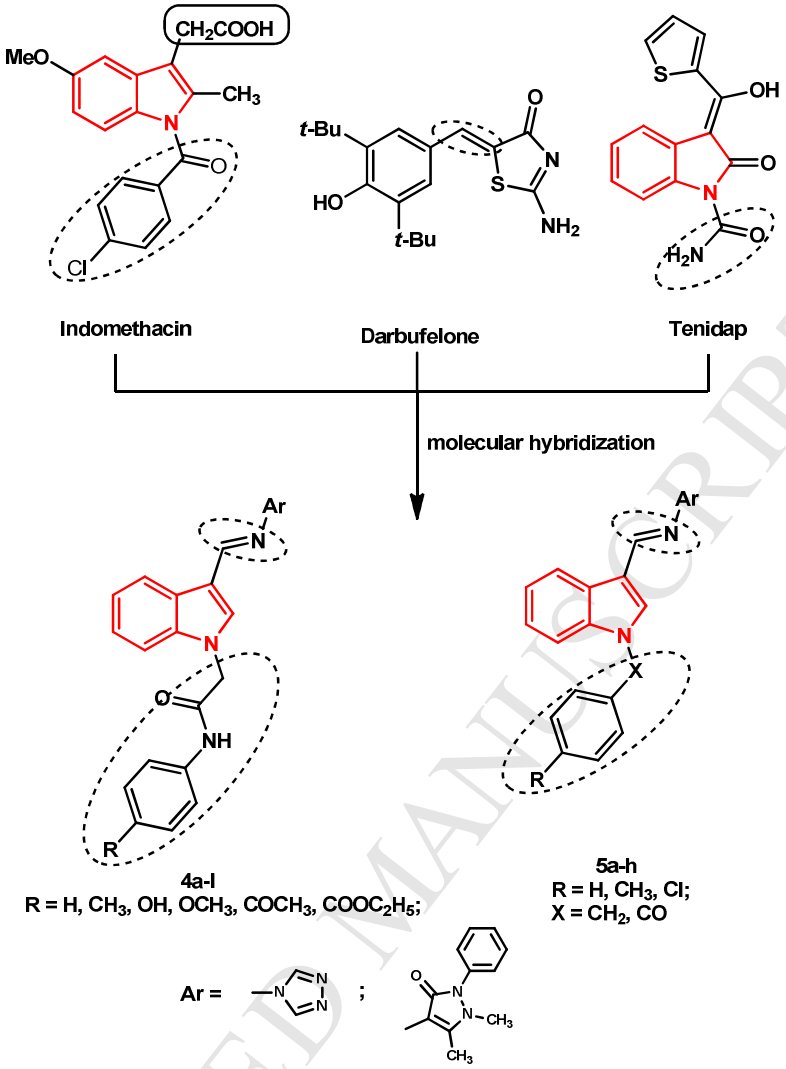
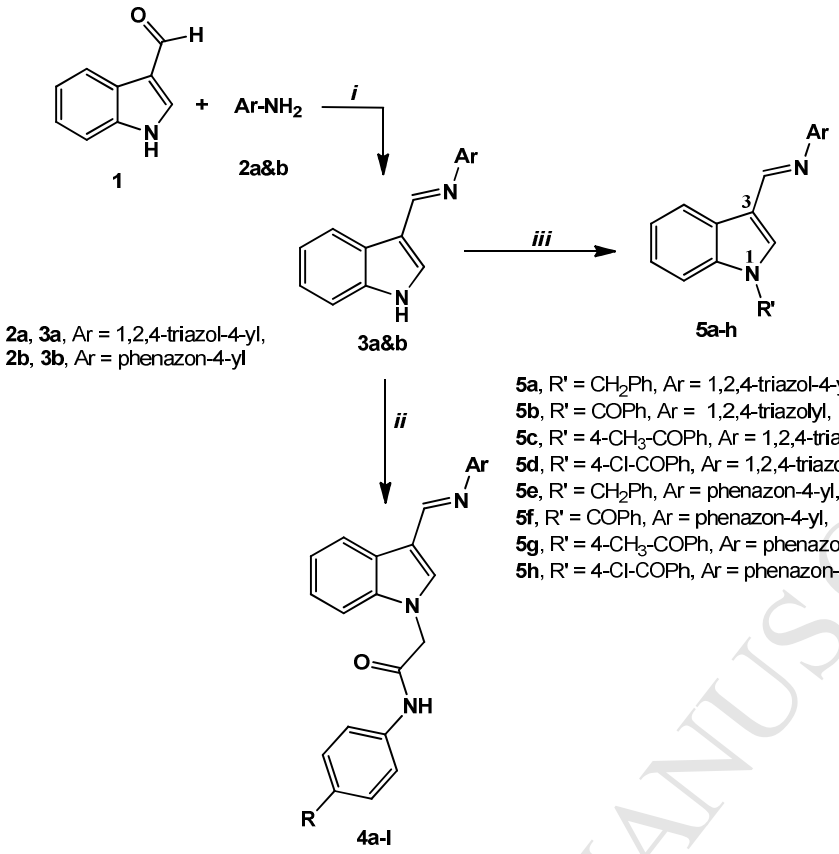


Figure 2



5a, R' = CH_2Ph, Ar = 1,2,4-triazol-4-yl,
5b, R' = COPh, Ar = 1,2,4-triazolyl,
5c, R' = 4-CH_3-COPh, Ar = 1,2,4-triazolyl,
5d, R' = 4-Cl-COPh, Ar = 1,2,4-triazolyl,
5e, R' = CH_2Ph, Ar = phenazon-4-yl,
5f, R' = COPh, Ar = phenazon-4-yl,
5g, R' = 4-CH_3-COPh, Ar = phenazon-4-yl,
5h, R' = 4-Cl-COPh, Ar = phenazon-4-yl

4a, R = H, Ar = 1,2,4-triazol-4-yl,
4b, R = CH_3, Ar = 1,2,4-triazol-4-yl,
4c, R = OH, Ar = 1,2,4-triazol-4-yl,
4d, R = OCH_3, Ar = 1,2,4-triazol-4-yl,
4e, R = $COCH_3$, Ar = 1,2,4-triazol-4-yl,
4f, R = COOEt, Ar = 1,2,4-triazol-4-yl,
4g, R = H, Ar = phenazon-4-yl,
4h, R = CH_3, Ar = phenazon-4-yl,
4i, R = OH, Ar = phenazon-4-yl,
4j, R = OCH_3, Ar = phenazon-4-yl,
4k, R = $COCH_3$, Ar = phenazon-4-yl,
4l, R = COOEt, Ar = phenazon-4-yl

Scheme 1. Reagents and conditions: ***i)*** abs. EtOH, reflux, 5-7 h; ***ii)*** 4-R-$PhCOCH_2Cl$, NaH, DMF, R.T., 24 h; ***iii)*** R'-Cl, NaH, DMF, R.T., 24 h.

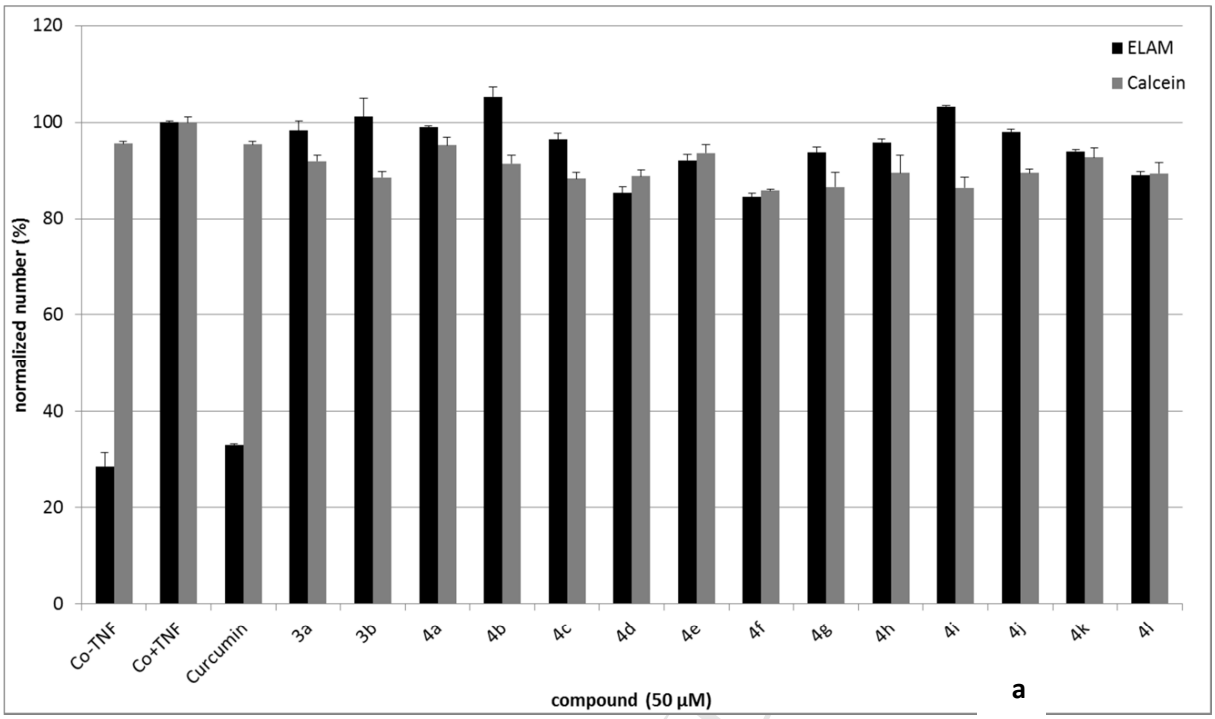
Table 1

Compound no	IC_{50}[a] (μM)		COX-2 SI[b]	IC_{50} (μM)[a]
	COX-1	COX-2		5-LOX
Indomethacin	0.63	11.36	0.055	n.d.
Celebrex	7.59	1.54	4.92	n.d.
Quercetin	n.d.	n.d.	n.d.	5.96
5a	9.21	2.01	4.58	7.41
5b	10.41	2.19	4.75	6.54
5c	7.89	0.98	8.05	6.54
5d	8.65	1.23	7.03	8.11
5e	11.23	2.61	4.30	5.98
5f	7.65	1.06	7.21	7.54
5g	10.8	2.23	4.84	5.78
5h	9.25	1.74	5.31	9.11

[a]The *in-vitro* test compound concentration required to produce 50% inhibition of COX-1 or COX-2. potato 5-LOX\soya bean 15 LOX assay kit, The result (IC_{50}, μM) is the mean of two determinations acquired using an ovine COX-1/ COX-2 assay Kit (Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.

[b]*In Vitro* COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

n.d. = not determined.


120
100
80
60
40
20
0
normalized number (%)
ELAM
Calcein
Co-TNF
Co+TNF
Curcumin
3a
3b
4a
4b
4c
4d
4e
4f
4g
4h
4i
4j
4k
4l
compound (50 µM)
a

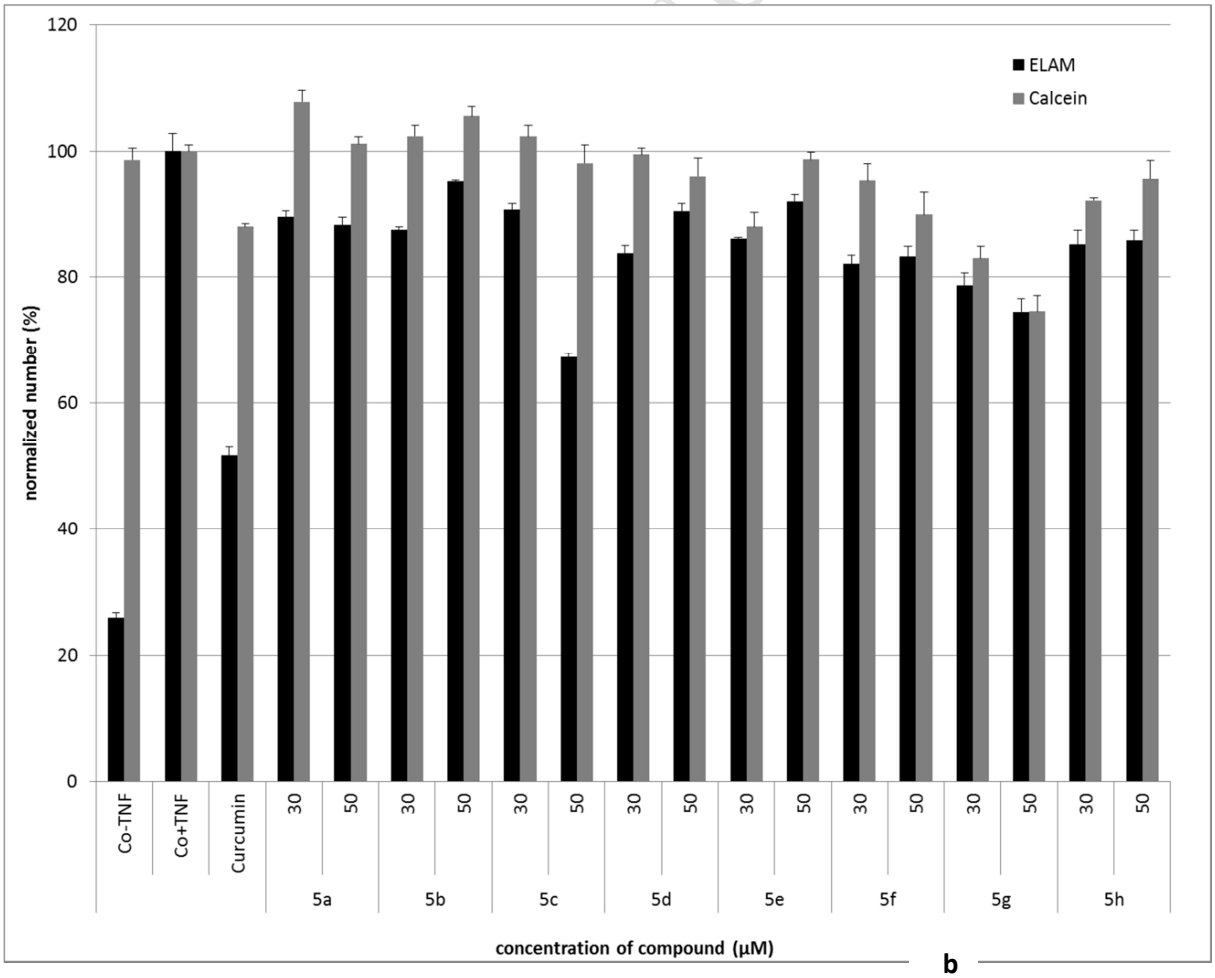

120
100
80
60
40
20
0
normalized number (%)
ELAM
Calcein
Co-TNF
Co+TNF
Curcumin
30
50
5a
5b
5c
5d
5e
5f
5g
5h
concentration of compound (µM)
b

Figure 3a, b

Table 2

Compound	CEM	MCF7	HeLa	BJ
3a	>50	>50	9.9 ± 1.7	>50
3b	>50	>50	>50	>50
4a	>50	>50	>50	>50
4b	>50	>50	>50	>50
4c	>50	>50	>50	>50
4d	>50	>50	>50	>50
4e	>50	>50	>50	>50
4f	>50	>50	>50	31.3 ± 0.6
4g	>50	>50	>50	>50
4h	42.0 ± 3.0	>50	>50	49.0 ± 1.7
4i	>50	>50	>50	50.0 ± 0.0
4j	50.0 ± 0.0	>50	>50	50.0 ± 0.0
4k	42.9 ± 8.4	>50	>50	>50
4l	29.7 ± 4.2	38.1 ± 1.3	24.8 ± 3.7	50.0± 0.0

Table 3

Compound no	COX2	5-LOX
	ΔG_{bind} best (kcal/mol)	ΔG_{bind} best (kcal/mol)
5a	-8.9	-7.4
5b	-9.3	-8.0
5c	-8.8	-7.9
5d	-8.7	-8.0
5e	-7.7	-6.9
5f	-7.7	-7.5
5g	-6.4	-6.9
5h	-5.8	-7.9

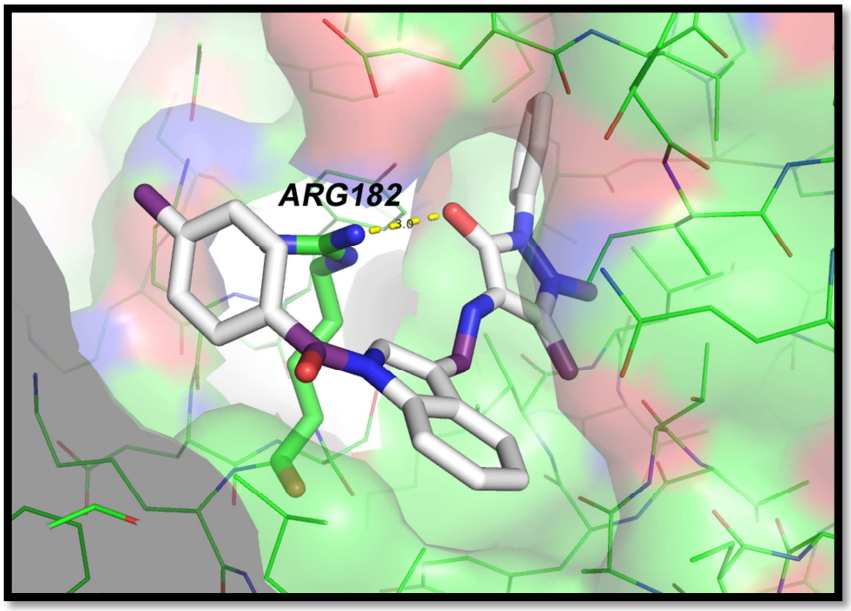


Figure 4

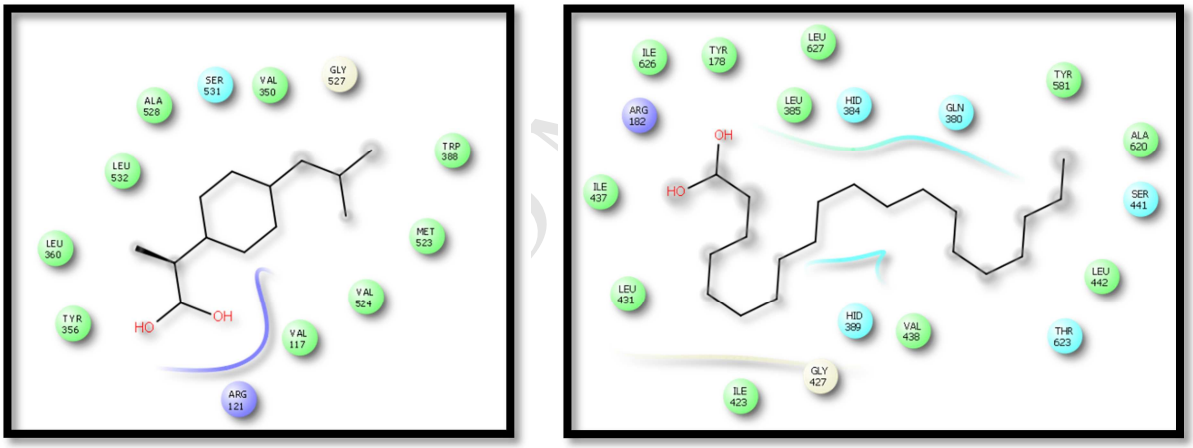


Figure 5

Highlights:

- *N*-substituted indole derivatives **4a-l** and **5a-h** were synthesized.
- Anti-inflammatory activity was evaluated.
- Compounds **5a-h** showed COX-2 inhibitory activity (IC_{50} = 0.98-2.61 µM).
- IC_{50} against 5-LOX for compounds **5e** & **5g** was 5.98 µM and 5.78 µM.
- Docking study for compounds **5a-h** was performed.